40 Autosomal SNP Loci Typed for U.S. African American, Caucasian, and Hispanic Samples Using Electrospray Ionization Mass Spectrometry

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Populations: U.S. Caucasian, African American, and Hispanic

Keywords: single nucleotide polymorphism; DNA; SNP; autosomal markers; mass spectrometry

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Abstract

A set of 194 self-described U.S. African American, Caucasian, and Hispanic samples was typed for 40 autosomal SNP markers intended for human identification purposes. Genotyping was performed on an automated commercial electrospray ionization time-of-flight (ESI-TOF) mass spectrometer, the PLEX-ID. The 40 SNP markers were amplified in eight unique 5plex PCRs, desalted, and resolved based on amplicon mass. The assay was found to be robust and capable of genotyping the 40 SNP markers consuming approximately 4 nanograms of template per sample. For each of the three U.S. sample groups, allele frequencies, heterozygosities, and p-values were calculated. The combined random match probabilities for the 40 SNP assay ranged from 10^{-16} to 10^{-21}.

Introduction

The application of autosomal single nucleotide polymorphisms (SNP) markers for human identification has been addressed in the forensic community (1-4). Single nucleotide polymorphisms may be of utility when working with highly degraded DNA because they can be assayed with very small PCR amplicons. Over the past 10 years various SNP assays and candidate marker panels have been described (5-10). One set of interest is a panel of 40 autosomal SNP markers intended as a universal individual identification panel. These markers were selected for high heterozygosity and low F_{st} values in studies of 40 populations to complement CODIS STR loci (8). Initially these markers were screened and typed for world populations by singleplex TaqMan-based assays. More recently, there have been attempts to develop multiplex assays for typing the 40 SNP panel (11). One of these is the PLEX-ID SNP40 comprised of 8 unique 5plex PCRs developed by Abbott Molecular. The PLEX-ID instrument platform is a commercial electrospray ionization mass spectrometer capable of automated analysis of short PCR amplicons (less than 140 bp) generated by proprietary assays (see SNP40, mtDNA 2.0). The instrument desalts each PCR reaction through the use of magnetic bead chemistry and injects the desalted PCR reaction into the mass spectrometer. The peaks are separated and resolved based on time-of-flight analysis. With the emerging development of ultra-high throughput sequencing applied to forensics it will be more commonplace to utilize these 'core' SNP maker sets. Here we report the assay performance and allele frequencies for a subset of our NIST U.S. population samples.

Methods

For this study samples (n = 194) were selected from three population groups representative of major population segments in the United States (African Americans = 74, Caucasians = 75 and Hispanic = 45).

Whole blood with anonymous identifiers and self-described ancestry was obtained from commercial blood banks (Interstate Blood Bank, Memphis, TN) (Millennium Biotech, Fort Lauderdale, FL). Blood samples were subjected to bulk DNA extraction using a modified salt-out

procedure as described previously (12). DNA concentrations in extracts were determined using Quantifiler Human DNA Quantification kit (Life Technologies, Carlsbad, CA) on an Applied Biosystems model 7500 (Life Technologies, Carlsbad, CA) real-time PCR instrument. Quantification values were then used to normalize all DNA extracts to a final concentration of 0.1 ng/µL for PCR amplification. All samples were previously examined with 15 autosomal short tandem repeats and the amelogenin sex-typing marker using the AmpFlSTR Identifiler kit (Applied Biosystems, Foster City, CA) to verify that each sample was unique (13).

SNP Typing

The 40 SNP markers typed by the PLEX-ID SNP40 assay were previously selected and characterized on multiple world populations (8). Table 1 contains a summary of the 40 SNP markers typed in the assay: dbSNP reference SNP (rs) number, nucleotide position (according to the Human February 2009 (GRCh37/hg19) assembly), chromosomal band, and physical distance from adjacent markers located on the same chromosome.

PCR amplification was performed as recommended by the manufacturer by adding a total of 0.5 ng in a 5 µL volume of template DNA to each of eight wells in a column of a pre-fabricated SNP40 assay plate (Abbott Molecular, Des Plaines, IL). Template DNA was added to each well by using a pipette tip to pierce the foil seal covering the well to which sample was added. On each 96-well plate, ten unique templates were run in parallel with a no-template control and a positive amplification control, 9947a DNA, (Promega Corp., Madison, WI) at 0.1 ng/µL. After template addition, the PCR plate was re-sealed using PCR Foil seals (Abbott Molecular, Des Plaines, IL) on an ALPS 50V Heat Sealer (ThermoFisher Scientific, Waltham, MA) by compressing the foil seal and PCR plate for four seconds at 180 °C. The prepared 96-well plate was then briefly centrifuged and placed in a Mastercycler ProS thermal cycler (Eppendorf AG, Hamburg, Germany) for thermal cycling with the following program: initial denaturation at 96 °C for 10 min; 40 cycles of denaturation at 96 °C for 20 seconds, annealing at 58.5 °C for 2 min, and extension at 72 °C for 10 s; followed by a final extension step at 72 °C for 4 min and a 4 °C hold.

Following PCR amplification the 96-well plate was briefly centrifuged and placed in the input stacker of the PLEX-ID instrument for automated desalting and mass determination as per manufacturer's recommended procedure.

PCR products were purified using a proprietary magnetic bead chemistry to remove salts, enzymes, unincorporated nucleotides, and any other PCR components which might interfere with collection of mass spectra. Purified PCR product was eluted in a buffer containing two peptide standards with masses of 727.4 Da and 1347.7 Da which act as calibrants to facilitate data processing. The electrospray ionization source operates in negative mode at approximately -4000 V (depending on the individual instrument's tuning parameters which are not user configurable) and 300 °C. PCR products were sprayed into the ionization source at a flow rate of 280 µL per hour with dry compressed air used as a countercurrent to aid in analyte desolvation. The time-of-flight analyzer collects 5000 scans per second, for a period of approximately 28 seconds. Masses were resolved based on differences in time elapsed to traverse the flight tube due to mass-to-charge ratio (*m/z*). Resultant mass spectra were processed by proprietary software which performs several steps to produce a background-subtracted, deconvolved representation of the mass spectral data as if only the singly charged mass peak were detected, with mass (Daltons) on the x-axis and signal strength (arbitrary units) on the y-axis. Successfully detected masses were stored in a table which resides in the Ibis Track database. The resulting mass spectra were inspected visually in IbisTrack software and any masses not correctly assigned by the software were manually added or deleted.

Following review, genotypes for each sample group were exported from IbisTrack to Excel for formatting and further analysis with Power Marker v3.25 and Arlequin software v3.5.1.2 (14,15). Allele frequencies, expected and observed heterozygosity values, and p-values (Fisher's exact test for Hardy-Weinberg equilibrium) for each marker were calculated for the three U.S. sample groups. The combined random match probabilities (RMP) for each sample were calculated using Excel.

Results

Figure 1 illustrates an example mass spectrum obtained from this study. Each spectrum contains the products of a 5plex PCR reaction. Eight separate 5plex reactions were run per sample requiring approximately 4 ng of DNA template per sample. Four signal peaks are typically observed for heterozygous loci, two forward and two reverse strands of DNA (see markers rs2272998 and rs445251 in Figure 1). Only two signals peaks are observed for homozygous loci (see markers rs6591147, rs321198, and rs3780962). Of the 194 samples examined in this study, incomplete or partial genotypes were observed for 21 loci (21/7760 = 0.27%). Ten of the failures were due to data not transferring to the PLEX-ID server due to a communication error. The remaining incomplete genotypes coincided with amplification reactions with that exhibited poor signal over the entire 5plex. This may have been due to inefficient PCR or desalting in those specific amplification reactions. There was no evidence of a single locus dropping out due to underlying SNPs that would affect PCR primer binding.

The genotype data for the 194 samples was evaluated for the following parameters: allele frequencies, observed heterozygosity, expected heterozygosity, and p-value. Table 2 contains these parameters for each of the U.S. sample groups.

The combined RMP match probability for each sample was calculated based on the determined allele frequencies for the corresponding sample group. Table 3 lists the median, minimum, and maximum combined RMP for each of the U.S. sample groups.

Discussion

A total of 6 of the 120 tests (40 loci x 3 sample groups) for Hardy-Weinberg equilibrium indicated a deviation from the expected result. It can be expected to observe approximately 5%, or 6 out of 120, of the comparisons to deviate from Hardy-Weinberg equilibrium (16,17). Those p-values significant at the 95% confidence level are those less than 0.05 (5%) and bolded in Table 2. Three were observed in Caucasian samples (rs1019029, rs1358856, and rs6811238),

two in African Americans (rs1523537 and rs447818) and one in the Hispanic data set (rs13182883). Note that the Bonferroni correction of probability for each population would have been 0.05/40 = 0.00125 and only the SNP marker rs1019029 would still be considered significant.

Typically the minimum number of samples needed to provide a robust estimate for allele frequencies with loci containing 5 to 15 alleles is 100 to 150 samples for each population (18). Since we are measuring bi-allelic markers in this study that only have three possible genotypes (AA, BB, or AB), a smaller number of samples should be sufficient—provided that a minimum allele frequency of 5/2N is utilized (19). An examination of the data for each sample group in Table 2 did not find any frequency measurements (out of the 360 total) below the 5/2N threshold.

In the Caucasian sample group the SNP marker rs1019029 exhibited a low p-value (< 0.0001) as well as a high observed heterozygosity of 0.733. The same marker gave an observed heterozygosity of 0.472 by Pakstis et al. over the 40 populations examined (8). An additional review of the mass spectral data did not reveal an obvious error with the genotyping assay. The high heterozygosity was not observed in the African American or Hispanic sample groups, suggesting that testing additional Caucasian samples and/or an alternate typing method would be needed to confirm this result.

The median combined RMP across the 3 U.S. sample groups was approximately 2.73×10^{-18} with a minimum of 2.39×10^{-16} (African America sample) and maximum of 2.86×10^{-21} (Hispanic sample). Pakstis et al. reported an average RMP across 40 populations of 10^{-16} with a range of 2.02×10^{-17} to 1.29×10^{-13} (8).

We found the PLEX-ID instrument and SNP40 assay to be a robust and automated method to type SNP markers. The time required to genotype 40 SNPs for 10 samples from start to finish (PCR to amplicon detection) was approximately 4.5 hours. The average time required to review

a plate (10 samples plus positive and negative controls) in the IbisTrack software was approximately 15 minutes. The allele frequencies calculated for the U.S. sample groups were found to be in agreement with published values with the possible exception of rs1019029. The allele frequencies are the first derived from the NIST U.S. populations sample set for this panel of 40 SNP markers intended as a universal panel for individual identification (8).

Access to the data: Genotyping results are available at: http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm.

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Tables

Table Captions

Table 1. Information for the 40 autosomal SNP loci examined in this study sorted by chromosome position. Chromosome positions were determined using the UCSC Genome Browser using Human Feb. 2009 (GRCh37/hg19) assembly.

Table 2. Allele frequencies observed for the 3 U.S. sample groups listed by SNP locus. Each SNP is identified with the corresponding dbSNP rs number. The format for each allele is listed in the line above the frequency. Example rs10092491 [C/T] where A = C and B = T. The p-values less than 5% for the markers rs1019029, rs1358856, rs6811238, rs1523537, rs447818 and rs13182883 are bolded.

Table 3. Summary of Random Match Probabilities calculated for the three sample groups. The median values, minimum and maximum observed RMPs for each sample group are listed.

Table 1 – The 40 SNP Markers

Marker	Chr	Nucleotide position	Chromosomal Band	Physical distance from adjacent marker (nt)
rs7520386	1	14,155,402	1p36.21	n/a
rs560681	1	160,786,670	1q23.3	146,631,268
rs1109037	2	10,085,722	2p25.1	n/a
rs12997453	2	182,413,259	2q31.3	172,327,537
rs6444724	3	193,207,380	3q29	n/a
rs279844	4	46,329,655	4p12	n/a
rs13134862	4	76,425,896	4q21.1	30,096,241
rs1554472	4	157,489,906	4q32.1	81,064,010
rs6811238	4	169,663,615	4q32.3	12,173,709
rs13182883	5	136,633,338	5q31.2	n/a
rs7704770	5	159,487,953	5q33.3	22,854,615
rs315791	5	169,735,920	5q35.1	10,247,967
rs338882	5	178,690,725	5q35.3	8,954,805
rs13218440	6	12,059,954	6p24.1	n/a
rs1336071	6	94,537,255	6q16.1	82,477,301
rs1478829	6	120,560,694	6q22.31	26,023,439
rs1358856	6	123,894,978	6q22.31	3,334,284
rs2503107	6	127,463,376	6q22.33	3,568,398
rs447818	6	145,868,996	6q24.3	18,405,620
rs2272998	6	148,761,456	6q24.3	2,892,460
rs214955	6	152,697,706	6q25.2	3,936,250
rs1019029	7	13,894,276	7p21.2	n/a
rs321198	7	137,029,838	7q33	123,135,562
rs10092491	8	28,411,072	8p21.1	n/a
rs3780962	10	17,193,346	10p13	n/a
rs1410059	10	97,172,595	10q24.1	79,979,249
rs740598	10	118,506,899	10q25.3	21,334,304
rs6591147	11	105,912,984	11q22.3	n/a
rs10488710	11	115,207,176	11q23.3	9,294,192
rs1058083	13	100,038,233	13q32.3	n/a
rs1821380	15	39,313,402	15q14	n/a
rs7205345	16	7,520,254	16p13.3	n/a
rs9951171	18	9,749,879	18p11.22	n/a
rs7229946	18	22,739,001	18q11.2	12,989,122
rs985492	18	29,311,034	18q12.1	6,572,033
rs445251	20	15,124,933	20p12.1	n/a
rs2567608	20	23,017,082	20p11.21	7,892,149
rs1523537	20	51,296,162	20q13.2	28,279,080
rs2073383	22	23,802,171	22q11.23	n/a
rs987640	22	33,559,508	22q12.3	9,757,337

Table 2 – Allele Frequencies for 3 U.S. Population Groups

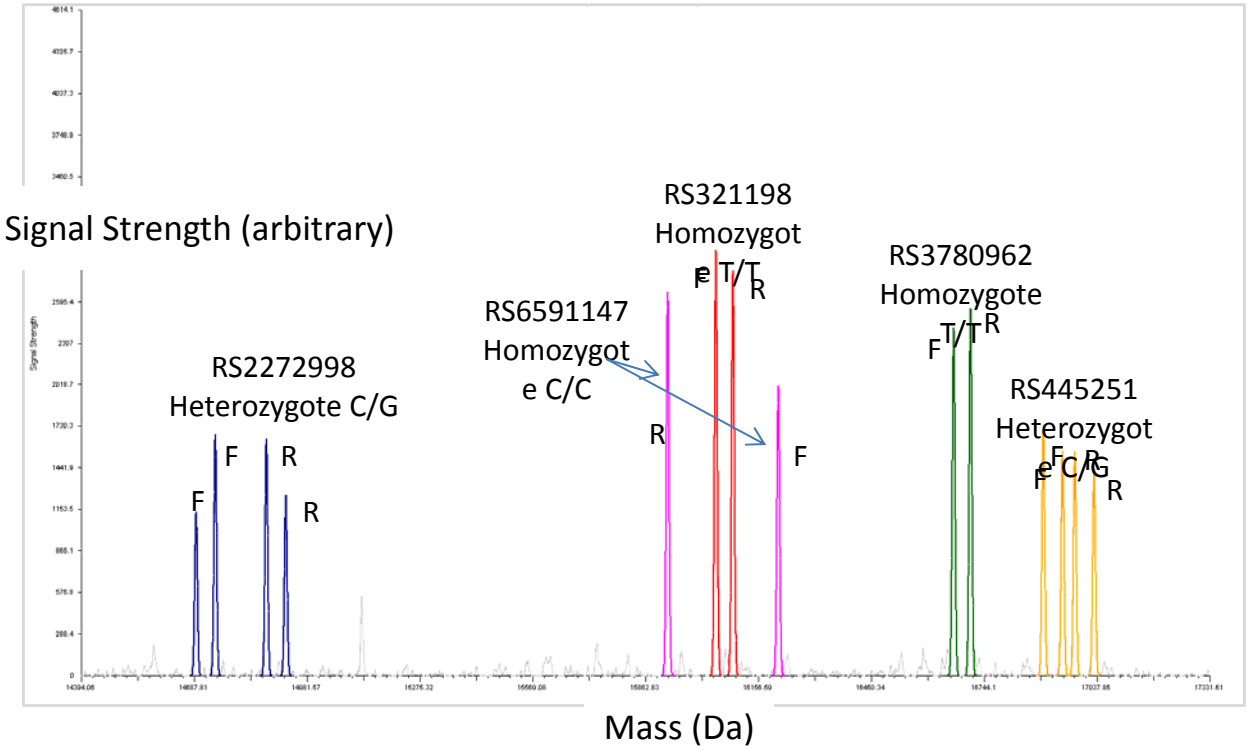
	Afr Amer	Cauc	Hisp	Afr Amer	Cauc	Hisp	Afr Amer	Cauc	Hisp	Afr Amer	Cauc	Hisp	Afr Amer	Cauc	Hisp
	rs10092491 [C/T]			rs1019029 [C/T]			rs10488710 [C/G]			rs1058083 [A/G]			rs1109037 [A/G]		
He	0.503	0.494	0.485	0.500	0.503	0.485	0.496	0.477	0.490	0.501	0.474	0.468	0.503	0.503	0.506
Hob	0.575	0.467	0.489	0.479	0.733	0.578	0.554	0.427	0.467	0.527	0.413	0.545	0.507	0.547	0.378
HoA	0.479	0.567	0.600	0.459	0.487	0.400	0.561	0.613	0.589	0.466	0.380	0.364	0.514	0.513	0.500
HoB	0.521	0.433	0.400	0.541	0.513	0.600	0.439	0.387	0.411	0.534	0.620	0.636	0.486	0.487	0.500
p-value	0.1546	0.6288	1.0000	0.6425	**0.0001**	0.2150	0.2272	0.4631	0.7510	0.6435	0.3191	0.3400	1.0000	0.4956	0.1320
	rs12997453 [A/G]			rs13134862 [A/G]			rs13182883 [A/G]			rs13218440 [A/G]			rs1336071 [A/G]		
He	0.441	0.494	0.463	0.492	0.464	0.475	0.503	0.488	0.502	0.462	0.417	0.497	0.458	0.494	0.475
Hob	0.486	0.600	0.444	0.419	0.560	0.400	0.432	0.427	0.318	0.438	0.373	0.556	0.397	0.440	0.356
HoA	0.324	0.433	0.356	0.426	0.360	0.378	0.486	0.413	0.455	0.356	0.293	0.433	0.349	0.433	0.378
HoB	0.676	0.567	0.644	0.574	0.640	0.622	0.514	0.587	0.545	0.644	0.707	0.567	0.651	0.567	0.622
p-value	0.4391	0.1040	1.0000	0.1492	0.0840	0.3470	0.1515	0.3523	**0.0230**	0.6126	0.4118	0.5650	0.2884	0.3453	0.1140
	rs1358856 [A/C]			rs1410059 [C/T]			rs1478829 [A/T]			rs1523537 [C/T]			rs1554472 [C/T]		
He	0.503	0.503	0.499	0.480	0.502	0.497	0.500	0.486	0.481	0.477	0.468	0.493	0.502	0.502	0.503
Hob	0.568	0.640	0.533	0.405	0.467	0.467	0.486	0.467	0.467	0.365	0.467	0.400	0.459	0.547	0.533
HoA	0.514	0.507	0.444	0.608	0.473	0.567	0.541	0.593	0.611	0.385	0.367	0.422	0.527	0.473	0.467
HoB	0.486	0.493	0.556	0.392	0.527	0.433	0.459	0.407	0.389	0.615	0.633	0.578	0.473	0.527	0.533
p-value	0.3532	**0.0222**	0.7660	0.2234	0.4888	0.7510	0.8134	0.8144	1.0000	**0.0261**	0.7980	0.2000	0.4775	0.3641	0.7560
	rs1821380 [C/G]			rs2073383 [C/T]			rs214955 [A/G]			rs2272998 [C/G]			rs2503107 [A/C]		
He	0.463	0.483	0.502	0.498	0.490	0.502	0.496	0.503	0.493	0.492	0.468	0.490	0.479	0.490	0.493
Hob	0.554	0.480	0.511	0.595	0.467	0.556	0.608	0.520	0.568	0.446	0.493	0.467	0.562	0.573	0.489
HoA	0.358	0.400	0.456	0.554	0.580	0.544	0.561	0.487	0.420	0.426	0.367	0.411	0.610	0.580	0.578
HoB	0.642	0.600	0.544	0.446	0.420	0.456	0.439	0.513	0.580	0.574	0.633	0.589	0.390	0.420	0.422
p-value	0.0804	1.0000	1.0000	0.1058	0.8075	0.5460	0.0614	0.8210	0.3590	0.3412	0.6295	0.7660	0.1675	0.0947	1.0000
	rs2567608 [A/G]			rs279844 [A/T]			rs315791 [A/C]			rs321198 [C/T]			rs338882 [C/T]		
He	0.503	0.503	0.497	0.501	0.498	0.506	0.496	0.503	0.481	0.488	0.496	0.505	0.500	0.503	0.502
Hob	0.548	0.560	0.422	0.554	0.493	0.600	0.466	0.520	0.556	0.500	0.466	0.378	0.507	0.560	0.422
HoA	0.479	0.520	0.567	0.466	0.553	0.500	0.562	0.513	0.611	0.588	0.562	0.522	0.459	0.507	0.544
HoB	0.521	0.480	0.433	0.534	0.447	0.500	0.438	0.487	0.389	0.412	0.438	0.478	0.541	0.493	0.456
p-value	0.3494	0.3567	0.3770	0.4816	1.0000	0.2420	0.6339	0.8214	0.3430	0.8141	0.6342	0.1700	0.8112	0.2645	0.3640
	rs3780962 [C/T]			rs445251 [C/G]			rs447818 [A/G]			rs560681 [A/G]			rs6444724 [C/T]		
He	0.500	0.498	0.505	0.477	0.490	0.485	0.496	0.490	0.497	0.415	0.433	0.425	0.494	0.496	0.505
Hob	0.541	0.493	0.444	0.473	0.452	0.489	0.658	0.440	0.511	0.419	0.387	0.422	0.405	0.533	0.556
HoA	0.541	0.553	0.511	0.385	0.418	0.400	0.438	0.420	0.433	0.709	0.687	0.700	0.568	0.440	0.522
HoB	0.459	0.447	0.489	0.615	0.582	0.600	0.562	0.580	0.567	0.291	0.313	0.300	0.432	0.560	0.478
p-value	0.6343	1.0000	0.5570	0.8014	0.4720	1.0000	**0.0047**	0.4735	1.0000	1.0000	0.4299	1.0000	0.1564	0.6400	0.5500
	rs6591147 [C/T]			rs6811238 [G/T]			rs7205345 [C/G]			rs7229946 [A/G]			rs740598 [A/G]		
He	0.503	0.464	0.490	0.485	0.494	0.505	0.500	0.500	0.485	0.500	0.499	0.497	0.487	0.452	0.481
Hob	0.527	0.480	0.422	0.486	0.360	0.444	0.514	0.520	0.622	0.479	0.480	0.511	0.466	0.493	0.511
HoA	0.520	0.640	0.589	0.595	0.567	0.511	0.541	0.540	0.600	0.459	0.453	0.433	0.589	0.660	0.611
HoB	0.480	0.360	0.411	0.405	0.433	0.489	0.459	0.460	0.400	0.541	0.547	0.567	0.411	0.340	0.389
p-value	0.8144	0.7994	0.3610	0.8135	**0.0211**	0.5700	1.0000	0.6288	0.0700	0.6553	0.8186	1.0000	0.6048	0.3051	0.7620
	rs7520386 [A/G]			rs7704770 [A/G]			rs985492 [C/T]			rs987640 [A/T]			rs9951171 [A/G]		
He	0.503	0.500	0.493	0.480	0.490	0.499	0.503	0.496	0.497	0.503	0.488	0.449	0.490	0.503	0.502
Hob	0.466	0.573	0.578	0.486	0.440	0.477	0.541	0.480	0.511	0.473	0.507	0.578	0.459	0.560	0.600
HoA	0.507	0.540	0.578	0.608	0.580	0.557	0.514	0.440	0.433	0.480	0.413	0.333	0.419	0.493	0.544
HoB	0.493	0.460	0.422	0.392	0.420	0.443	0.486	0.560	0.567	0.520	0.587	0.667	0.581	0.507	0.456
p-value	0.6534	0.1666	0.3550	1.0000	0.4948	1.0000	0.5155	0.8242	1.0000	0.6496	0.8243	0.1150	0.6274	0.2435	0.2270

Table 3 – Combined Random Match Probabilities

Combined Random Match Probability	Afr. American	Caucasian	Hispanic
median	3.37E-18	2.53E-18	2.29E-18
min	2.15E-20	4.09E-21	2.86E-21
max	2.39E-16	4.76E-16	7.35E-17

Figure Legends

Figure 1 An example mass spectrum is shown. This specific example contains 3 homozygous (rs6591147, rs321198, rs3780962) and 2 heterozygous (rs2272998 and rs445251) markers. The forward and reverse strands for each PCR amplicons are highlighted. Note the mass interleaving of SNPs rs6591147 and rs321198.



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Abbreviations

PCR – polymerase chain reaction

STR - short tandem repeat

SNP – single nucleotide polymorphism

ESI-TOF - ionization time-of-flight

RMP – random match probability